Conchology of endangered freshwater pearl mussel: conservation palaeobiology applied to museum shells originating from northern Finland

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Abstract

Shells of Finnish freshwater pearl mussel (*Margaritifera margaritifera*) were analyzed by means of taphonomy and sclerochronology. The samples originated from two small rivers in northern Finland, River Kotioja and River Saukko-oja. Taphonomical analysis showed that the mussels died recently and that the shells were likely to be opened violently through pearl hunting. Internal shell growth increments were studied on annual basis. Annual shell growth increments showed life-long trends, attributable to factors internal to growth. The trends were removed individually from each series and the resulting time series were statistically compared within and between the two rivers. The temporal shell growth variability was distinctly similar within the datasets of both rivers, evidenced by inter-series correlations. This correlativity indicated shared growth response to external factors (*i.e.* climate and hydrology) and further justified the construction of mean river-specific shell growth chronologies, sclerochronologies. The construction of the chronologies was demonstrated by means of sclerochronological crossdating, indexing and chronology stripping. The two chronologies were compared by their growth variability visually and statistically. The comparison showed that (1) the amplitudes of the growth variations were not much different between the rivers, and, that (2) the two sclerochronologies could be linked in time domain. These results indicated that (1) the habitats of the two populations were insignificantly different owing to the environmental variability magnitude, and, that (2) the external control on the growth variability involved the same (*i.e.* regional) climatic factors regardless of the river.

Riassunto

Il bivalve dulcicolo *Margaritifera margaritifera* è una specie a rischio di estinzione in tutto il suo areale di distribuzione. Capire le relazioni ecologiche di questa specie con l'ambiente è essenziale per organizzarne la protezione. Informazioni sulla crescita del bivalve possono essere ricavate dall'esame delle strie di accrescimento, esaminate in sezione trasversale. Inoltre, l'analisi tafonomica può dare indicazioni su cause e tempi della morte degli individui. In questo lavoro, si esaminano due campioni di conchiglie provenienti dai fiumi Kotioja e Saukko-oja, nella Finlandia settentrionale, conservati nel Museo Finlandese di Storia Naturale. Allo scopo di mettere in luce la storia *pre* e *post mortem* degli esemplari, sono state condotte osservazioni tafonomiche e sclerocronologiche. L'identificazione degli incrementi interni di crescita è stata effettuata su base annua, separatamente per il materiale provenienti dai due fiumi. I dati tafonomici indicano che gli esemplari sono morti in modo violento e in tempi recenti. Frequenti fratture sulla parte dorso-posteriore sono interpretate come causate dall'apertura forzata delle valve, per la raccolta illegale di perle. La morte improvvisa e simultanea dei bivalvi è dimostrata anche dall'ultimo incremento di crescita, eccezionalmente stretto e corrispondente allo stesso anno fra i vari esemplari. Per il materiale di entrambe le località, sono stati messi in evidenza gli incrementi annui medi di crescita. La crescita delle conchiglie sembra essere avvenuta con lo stesso tipo di variabilità (annuale ed a più lungo termine) in entrambi i fiumi, suggerendo che i due ambienti hanno subito variazioni ambientali di ampiezza simile. Sulla base della letteratura, si può ritenere che il fattore primario che sta dietro la variabilità nella crescita sia la temperatura estiva. I dati sclerocronologici mostrano una correlazione statisticamente significativa fra le due popolazioni: ciò implica che esse possono essere correlate cronologicamente. Come prospettive future, si potrebbero raccogliere dati sclerocronologici secondo una più fitta maglia regionale, al fine di migliorare la conoscenza dell'ecologia della specie e la sua dipendenza dai fattori ambientali in fiumi diversi.

Introduction

Freshwater pearl mussel (*Margaritifera margaritifera*) is an endangered species, which has suffered from pearl hunters and aquatic contaminants (Bauer, 1986, 1988). Conservation plans and ecological study programs were conducted in several countries in order to enhance the potentiality of the species to survive 21st century threats (Young, 1991; Araujo & Ramos, 2001; Skinner, *et al.* 2003; Valovirta *et al.*, 2003). Increased knowledge of the habitat requirements and other ecological features of M. *margaritifera* is of great importance in the protection plans. In northern Europe, and especially in Finland (Valovirta *et al.*, 2003), the earlier threat was primarily pearl fishing while the modern threats are associated with the drainage of peatlands and silvicultural treatments that change sedimentation rates and water quality of the rivers. Negative factors include eutrophication, river dredging and the construction of submerged dams for the purposes of fishery. M. *margaritifera*

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was protected in Finland since 1955, but many of the populations are currently not reproducing due to poor water quality (Valovirta *et al.*, 2003).

Invaluable information about bivalve ecology can be obtained from conchological studies. *M. margaritifera* is a long-lived mussel, reaching a maximum life span of more than hundred years, and forms shell increments with an annual periodicity (Bauer, 1992; Mutvei *et al.*, 1994, 1996; Dunca, 1999; Ziuganov *et al.*, 2000; Dunca & Mutvei, 2001). Internal growth structures are visible in the cross-sections of the shells and serve as quantitative records that span over the life time of the individual. Shell growth increments can be used to infer the ecological requirements and environmental controls of different species. For example, Jones (1981) studied the internal growth increments of *Spisula solidissima* along the New Jersey coast and found that the growth variability indicated sea surface temperatures and success of juvenile recruitment. Tanabe (1988) used internal annual and microgrowth increments of *Phacosoma japonicum* for age and growth rate determinations in Japanese coastal area and presented a linkage between the increment growth and tidal rhythms. Seire *et al.* (1993) studied increment growth rates from cross-sections of *Macoma balthica* and their relationships to environmental factors and pollution along the southern shore of the Gulf of Finland. Dunca *et al.* (2005) studied the internal growth increments of *M. margaritifera* in Sweden and demonstrated the relationships between the growth, the summer temperatures and the aquatic pollution. Bivalves that grew in a given site or region were influenced by the same climatic and aquatic factors. Increments are thus expected to exhibit common growth signal and a number of individual growth series can be combined into one mean chronology that better reflects the population growth variability (Marchitto *et al.*, 2000; Helama *et al.*, 2006a).

Here we study the collection of shells of *M. margaritifera* that were previously collected along two rivers in northern Finland. The samples now belong to the collections of the Finnish Museum of Natural History, University of Helsinki. The usage of the museum or subfossil/fossil shells is of particular importance in the case of endangered species, such as *M. margaritifera*, to preserve the populations that still exist. Palaeoecological techniques were applied to the analysis of the historic skeletal remains of species that are threatened with extinction. Such a biological approach has been coined to as conservation palaeobiology by Flessa (2002).

Taphonomical analysis was performed to reveal the post-mortem and mortal-histories of shell specimens. Internal shell growth structures were examined to display the annual growth increments of the bivalves. Increment width records from individual specimens were constructed and combined into river-specific shell growth increment chronologies. The two rivers were compared by the sclerochronological statistics. Our sclerochronological aims were (1) to present two new shell increment chronologies for northern Finland, (2) to demonstrate the identification of the annual growth increments, (3) to examine the inter-annual to decade-scale behaviour of the shell growth and (4) to compare the river-specific records to seek the potential signature in growth for ecological and environmental characteristics of the habitats.

Material and methods

Shells of *Margaritifera margaritifera* (Linnaeus 1758) were collected from the proximity of two rivers in northern Finland. River Saukko-oja is situated in the municipality of Salla in south-eastern Lapland and River Kotioja runs in the municipality of Taivalkoski in Northern Ostrobotnia (**Fig. 1**). Fourteen shells were found at River Saukko-oja (July 27th 1984) and seven shells at River Kotioja (July 13th 1979) (**Tab. 1**). All the specimens were found as empty shells at the time of collection, lying on the surface sediments on the river sides. The exact year of death of the animals is thus unknown.
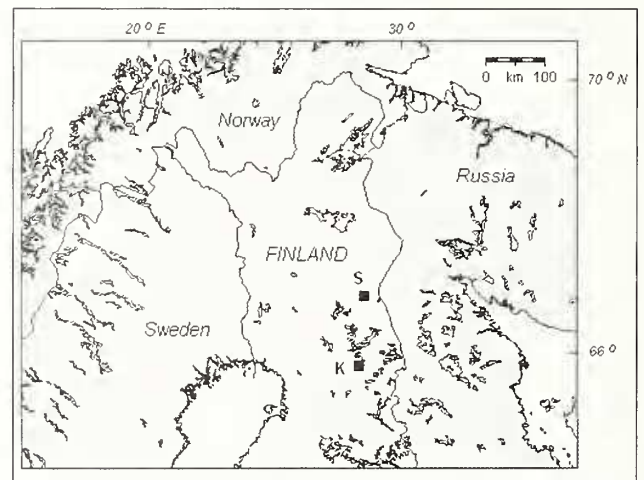

Fig. 1. Map showing the shell collection sites as squares, River Kotioja (K) and River Saukko-oja (S).

Fig. 1. Ubicazione dei siti di raccolta (quadrati) nei Fiumi Kotioja (K) e Saukko-oja (S).

Site\Statistics	Samples	Years	SD	ms	AR1
River Kotioja	7	24	0.201	0.253	− 0.311
River Saukko-oja	14	36	0.275	0.238	0.353

Tab. 1. Descriptive statistics for the sclerochronologies from River Kotioja and River Saukko-oja. Calculated statistics were the total samples size (Samples), temporal length of the chronology (Years) with at least four samples, standard deviation (SD), mean sensitivity (ms) and first order autocorrelation (AR1).

Tab. 1. Dati statistici descrittivi per le sclerocronologie dei Fiumi Kotioja e Saukko-oja: numero totale dei campioni (Samples), durata della cronologia (Years) con almeno quattro campioni, deviazione standard (SD), sensibilità della media (ms) e autocorrelazione di primo ordine (AR1).

Macroscopic and microscopic analyses of the shells

Specimens were studied to obtain an overview of their preservation. The stage of pre-mortem bioerosion and dissolution as well as post-mortem bioerosion, abrasion, disarticulation and fragmentation were examined through macroscopic inspection (see Nielsen, 2004). The purpose of this taphonomical analysis was to estimate the post-mortem and mortal-history the specimens.

In order to examine the internal shell growth structures, shells were cut, cross-sections of the cut surface polished and the annual growth lines observed under computer-integrated microscope system. Following the methods described by Dunca & Mutvei (2001), one valve of each specimen was cut from the umbo to the ventral margin perpendicular to the winter lines and along the axis of minimum growth. Complete growth records for most species are found along the axis of maximum growth. However, Dunca & Mutvei (2001) counted exactly the same number of increments in the axes of minimum and maximum growth sections with better visibility in the minimum growth section. The sections were ground (800 and 1200 grit metallographic grinding paper), polished (3 μm diamond paste) and then etched in Mutvei's solution at 37-40°C for ca. 25 min, carefully rinsed in de-ionized water and allowed to air-dry (Mutvei et al., 1996; Schöne et al., 2005). While the acetic acid dissolved the carbonate, glutaraldehyde fixated the organic matrix, and alcian blue stained and fixated the mucopolysaccharids and glucosamids in the shell. This treatment resulted in an excellent three-dimensional preservation of the growth structures with distinct, etch-resistant, blue-colored winter lines. Finally, annual growth increments were viewed under a reflective light binocular microscope and digitally photographed. Widths of all the increments were measured from the outer shell layer, with 1 μm precision, perpendicular to the growth lines.

Time series analyses - detecting the growth synchrony

Prerequisite of any sclerochronological time series analysis is the procedure called crossdating (Douglass, 1941; Fritts, 1976; Marchitto et al., 2000; Helama et al., 2006a). Due to common environmental and climatic forcing factors, the wide and narrow annual growth increments are expected to occur in synchrony. This synchrony may occur over large spatial scales and can be used to assign correct dates to shell increments. Therefore, the year-to-year variability in the growth rates may be synchronized over one specific site or over wider areas if the growth of the organisms reacted similarly to the shared climatic or aquatic factors. As individuals of the same species are expected to respond to external factors similarly, the theory of crossdating predicts that the synchrony between individuals is most likely to occur at species-level. Furthermore, averaging of a number of crossdated individual time series into one site and species-specific mean chronology is expected to emphasize the growth variations at the population level and it is advantageous to compare each individual growth series to the series of mean growth (master chronology) instead of single individuals (see Helama et al., 2006a). Previously, river-specific chronologies based on the annual growth increments of alive-collected *Margaritifera margaritifera* displayed positive correlations within and between sites in Sweden (e.g. Mutvei et al., 1994; Helama et al., 2006a).

Growth synchrony was examined herein using visual comparison and quantified by the Pearson product-moment correlation coefficient (r) (Holmes, 1983). Pearson correlation measures a statistical equation describing the linear relationship between two time series.

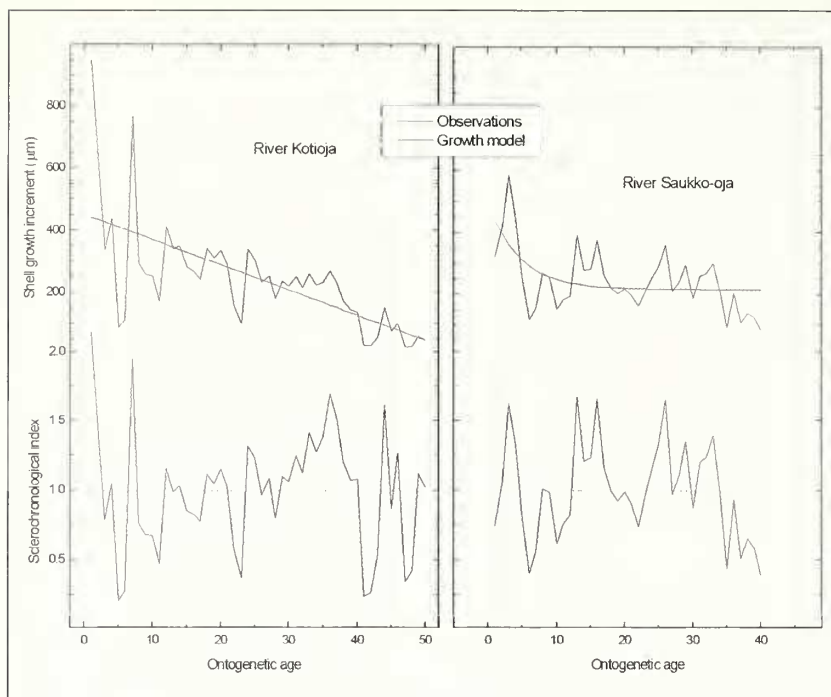## Time series analyses - growth trend modelling

Prior to actual crossdating, individual growth series were indexed by detrending the initial measurement series. Individual series of annual *M. margaritifera* shell growth increments exhibit a trend as a function of ontogenetic age of the organisms, demonstrated previously though the investigations of internal (Mutvei et al., 1994; Dunca, 1999; Dunca & Mutvei, 2001; Helama et al., 2006a) and shell surface (San Miguel et al., 2004) increments. Such a trend, commonly referred to as a 'growth trend', is largely due to ontogeny of the organism and, accordingly, target of removal prior to analysis of common growth signal (e.g. Fritts, 1976; Helama et al., 2004, 2006a). In the present study, growth trends were modelled using regression lines and modified negative exponential curves (Fritts et al., 1969; Fritts, 1976) and subsequently removed by dividing the observed annual growth value by the expected growth value of the modelled curve. Model with better fit was chosen as a final model of ontogeny (**Fig. 2**). Resulting ratio-based growth indices were dimensionless.

Growth indices of shell increments were crossdated using all available growth time series for each site. Site-specific chronologies were averaged using all crossdated series by arithmetic mean.
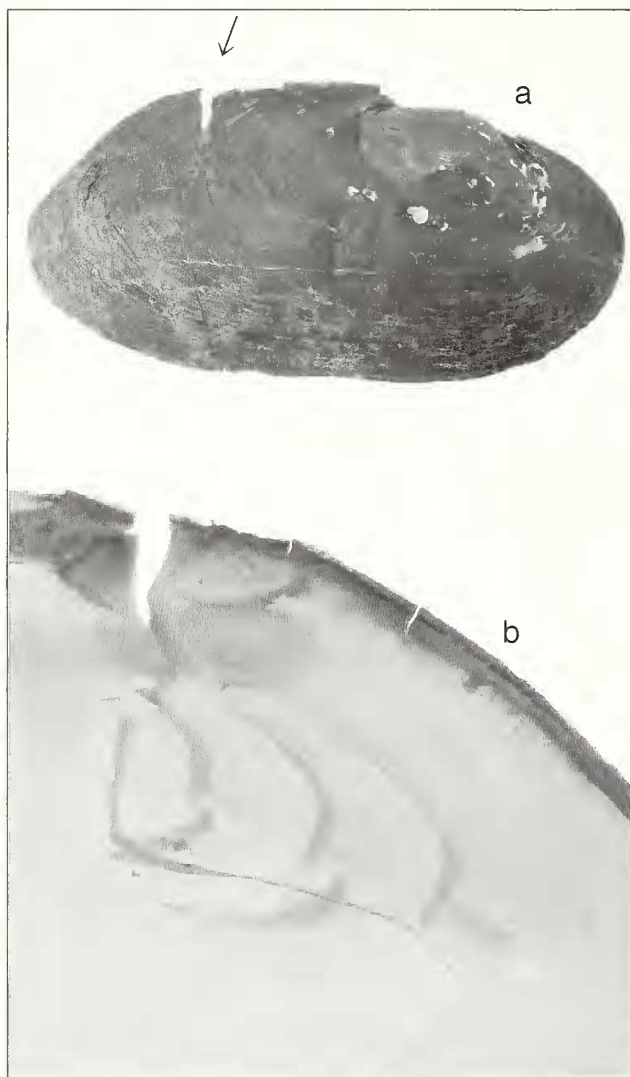
Sclerochronological statistics

Many aspects in the study of shell growth increments, i.e. sclerochronology, are highly similar in theoretical basis to the study of tree-rings, dendrochronology (Fritts, 1976). As a matter of fact, the aforementioned principles of crossdating and growth trend modelling were originally developed by dendrochronologists (Huntington, 1914; Douglass, 1941) and later adopted to sclerochronology during the past decades (Marchitto et al., 2000; Helama et al., 2006a). According to dendrochronological theory, the site characteristics can be detected from the behaviour of tree-ring series, that is, by means of tree-ring statistics (Fritts et al., 1965).

Statistics that can be related to ecological or environmental gradients are for example the measures of variability: standard deviation and mean sensitivity (Fritts et

Fig. 2. Growth trend modelling exemplified for two *Margaritifera margaritifera* (L.) shell increment series. Regression lines and modified negative exponential curves were fitted to initial series of increment width observations (upper plots). Modelled growth trends were removed by dividing the observed values by modelled values. Resulting dimensionless index series no longer exhibit long-term growth decline (lower plots).

Fig. 2. Modellizzazione dell'andamento di crescita esemplificato per gli incrementi di crescita in due conghiglie di *Margaritifera margaritifera* (L.). Le linee di regressione e le curve esponenziali negative modificate sono state adattate ai dati delle serie iniziali di incremento in ampiezza (grafici in alto). Gli andamenti di crescita modellizzati sono stati rimossi dividendo i valori osservati per i valori modellizzati. L'indice adimensionale risultante non mostra più il rallentamento di crescita a lungo termine (grafici in basso).

al., 1965; Fritts, 1976). Mean sensitivity (ms) was determined by Fritts (1976) as

$$ms = \frac{1}{n-1} \sum_{t=1}^{t=n-1} \left| \frac{2\left(x_{t+1} - x_t\right)}{x_{t+1} + x_t} \right|$$

where x is the tree-ring index (here, growth index of shell increments) of year t in the series possessing n tree-rings (here, increments). Compared to standard deviation, mean sensitivity measures the growth variation between consecutive years (i.e. variability at high frequencies). Standard deviation and mean sensitivity were used here in comparison of the results of River Saukko-oja and River Kotioja. In addition, the first order autocorrelations were calculated.

Results

Taphonomical analysis

All the shells were preserved in pristine condition with intact periostracum and major portions of ligament. Inner shell surface was unaffected by dissolution and other possible deteriorating processes. In contrast, the umbonal region was corroded to a severe extent, which probably took place during the life of the specimens. This was commonly seen in still living freshwater bivalves. Fractures were common next to the posterior muscular scar, cutting the dorso-posterior margin (**Fig. 3**). Also, the ventral margin contained sporadic fractures. A knife cutting the muscular meat and separating the valves from each others may have caused these fractures.

Overall, the excellent preservation of all shells suggested that they were collected alive recently, and have only shortly been transported in natural ways before they were unearthed for the museum collections. Previous studies have shown that the preservational condi-

Fig. 3. Photographs of the *Margaritifera margaritifera* shell showing the fracture (arrow) cutting the dorso-posterior margin (a), across the posterior muscular scar (b).

Fig. 3. Una conchiglia di *Margaritifera margaritifera* con frattura (freccia) sul margine postero-dorsale (a), e attraverso l'impronta muscolare posteriore (b).

tions of shells are not necessarily related to the stratigraphical age of the shells (e.g., Carroll *et al.*, 2003). However, the shells of *Margaritifera margaritifera* appeared to be recently collected alive before they became taphonomically altered by acidic rain water and bioturbation, e.g., by plant roots.

Annual growth increments

Widths of the annual growth increments were measured perpendicularly to winter lines (**Fig. 4 a**). The main difficulty of measuring the increments was to distinguish the actual winter lines from disturbance or potential reproduction lines that were sporadically observed between the winter lines. Misinterpretation of different growth breaks and corresponding growth lines would lead to shell increment time series with biased temporal control and over- or underestimated ontogenetic ages. These misinterpretations were avoided using the following observational criteria: (1) winter lines can be followed through the outer prismatic layer and penetrates into inner nacreous layers without disruption, (2) winter lines often cut the interface between the nacreous and prismatic layers as a small ripple (**Fig. 4 b**), (3) intra-annual growth lines (if visible) are considerably narrower alongside winter lines than the ones alongside disturbance lines, (4) disturbance lines are often associated with fractures penetrating from shell surface into prismatic (but not nacreous) layer (**Fig. 4 a**). These observational criteria were supported by the simultaneous crossdating of the shell growth increments (see below). Mean number of annual increments in the specimens from River Kotioja and River Saukko-oja were 19 and 29 respectively.

Growth synchrony between the shell growth indices

The growth synchrony between individuals was remarkable when the index series were aligned by their last identifiable growth increment. This implied that the shells collected from River Saukko-oja died within the same calendar year (**Fig. 5**). The same observation was made for shells from River Kotioja.

The widths of the last identifiable growth increments were relatively narrow in each sample, and since there could be seen no sign of winter lines in the uttermost part of the ventral margin in the cross-section of the shells, it was likely that all the studied mussels had stopped growing during the actual growing season.

Individual sample series of shell growth index series were compared separately with the master chronology (arithmetic mean of all other series) (**Fig. 5**). Statistical comparison supported the results from the visual comparison about the inter-series growth synchrony (**Figs. 5** and **6**). Judging from the relatively high correlations between the series in River Saukko-oja, it is likely that the mussels probably lived in a shared habitat thus not too far from each other. Growth synchrony appeared to be present although somewhat less evident in River
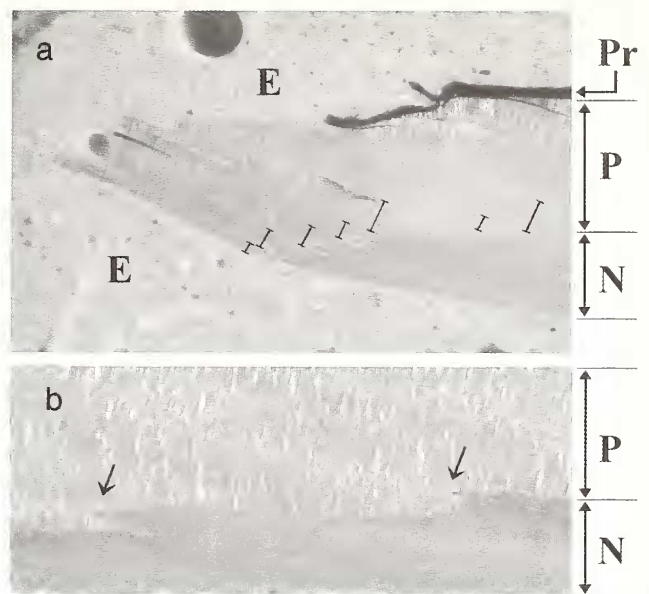

Fig. 4. Cross-section of the prismatic (P) and nacreous (N) layers from the ventral part of the shell with fragmentary periostracum (Pr), here surrounded by supporting epoxy (E). Annual increments were measured perpendicular (black bars) to the winter lines (a). Winter line cuts the interface between nacreous and prismatic layers typically with a small ripple (arrow) (b). The specimen originates from River Saukko-oja.

Fig. 4. Sezione trasversale inglobata in resina epossidica (E) dello strato prismatico (P) e madreperlaceo (N) nella regione ventrale della conchiglia, con porzioni di periostraco (Pr). Gli incrementi annuali di crescita sono stati misurati perpendicolarmente (barrette nere) alle linee invernali (a). Le linee invernali intersecano l'interfaccia tra lo strato madreperlaceo e quello prismatico, producendo (freccia) una tipica ondulazione (b). L'esemplare proviene dal Fiume Saukko-oja.

Kotioja (**Fig. 6**). According to crossdating, there were three shell growth increment series with correlation coefficient with master series below 0.4 in the River Kotioja population. We used this as an arbitrary level for acceptance of series into the chronology: the three series possessing low correlations with the master chronology were not included in the final, stripped, sclerochronology from River Kotioja (**Fig. 6**).

Parameters of growth behaviour

Shell increment statistics showed no significant difference between the site characteristics (**Tab. 1**). Standard deviation was somewhat higher for River Saukko-oja but the mean sensitivity was higher for River Kotioja. Both the standard deviation and mean sensitivity are measures of variability, but the mean sensitivity is expected to measure more effectively the year-to-year variability whereas standard deviation accounts for variability at all time scales. This implied that the inter-annual growth variability was somewhat more intense in River Kotioja, however, the overall growth variability was greater in River Saukko-oja. This may be, at least partly, due to the shorter length of individual sample series in River Kotioja dataset: long-period growth variations may not have been preserved in the chronology since the variations at timescales longer than segment lengths were removed in the growth trend modelling (see Cook *et al.*, 1995; Helama *et al.*, 2004). First-order autocorrelations were rather low and insignificant for
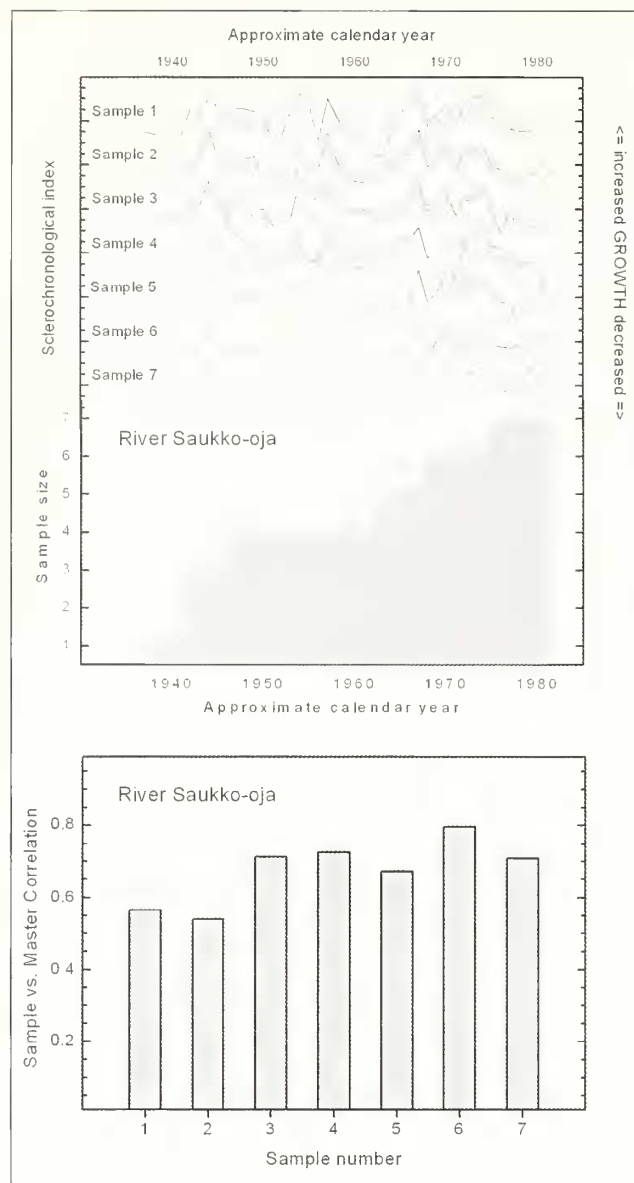
Fig. 5. Visual comparison of growth in individual sample series (thin black line) and master chronology (thick grey line) after growth trend modelling (Fig. 2). Temporal variation in sample size in River Saukko-oja chronology shown as grey area. Statistical comparison of growth in sample series and master chronology quantified by correlation coefficients (r) (histograms).

Fig. 5. Confronto visuale della crescita in serie individuali (linee sottili nere) e cronologia media (linee spesse grigie) dopo la modellizzazione dell'andamento di crescita (Fig. 2). La variazione temporale della dimensione degli individui rispetto alla cronologia del Fiume Saukko-oja è rappresentata dall'area grigia. L'istogramma mostra il confronto statistico fra la crescita individuale e la cronologia media attraverso il coefficiente di correlazione (r).

both chronologies, in fact, the River Kotioja chronology showed a negative autocorrelation.

Inter-river comparison

Sampled shells were provided to museum as dead specimens and thus the actual year of death was not known. As demonstrated earlier, however, individuals were believed to have died at same time in each river. Comparison between the two sclerochronologies could in this work reveal their relative temporal position. It seems conceivable that the population in River Kotioja would have died 10 years before the population in

River Saukko-oja. In this chronological position, the highs and lows of shell growth correlated significantly (**Fig. 7**). Correlation was evident with growth variations at inter-annual to decadal time scales. However, the preliminary nature of the obtained correlation should be emphasized. Inclusion of more samples would be preferred in the future work to elongate the chronologies temporally and to strengthen the growth signal in both river-specific chronologies to ensure estimated growth variations in both rivers.

Discussion

Information from taphonomy and sclerochronology

Museum collection of subfossil freshwater pearl mussel (*Margaritifera margaritifera*) shells was studied. The set of samples, originating from two small rivers, River Kotioja and River Saukko-oja in the northern Finland, was examined by means of taphonomy and sclerochronology. Previously, the conservational biology of the species have largely been based on morphometrical studies, in particular the relationships between the shell length and ontogenetic age (Bauer, 1991, 1992; Hastie *et al.*, 2000; San Miguel *et al.*, 2004). In the course of this study, we have demonstrated how carefully examined information from taphonomy and sclerochronology can be used for the purposes of conservation biology and ecology of the species. In the forthcoming sections, the
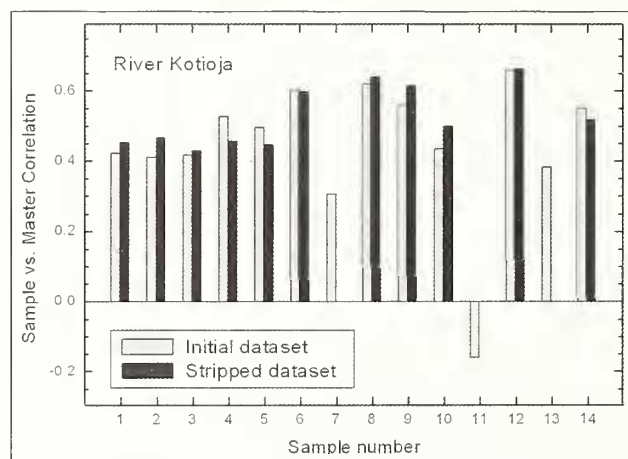
Fig. 6. Statistical comparison of growth in sample series and master chronology (arithmetic mean of all other series) quantified by correlation coefficients (r) (histograms) in River Kotioja. Sample series of initial dataset (grey histograms) having correlation lower than 0.4 (dotted horizontal line) with master chronology were removed (Samples 7, 11 and 13). Stripped dataset (black histograms) yielded master chronology with enhanced common growth signal, indicated by higher intercorrelations between the remaining samples series and master chronology (mean Sample vs. Master correlations for initial and stripped datasets were 0.445 and 0.526, respectively).

Fig. 6. Confronto statistico fra la crescita individuale ed i valori di crescita media, tramite il coefficiente di correlazione (r) nel Fiume Kotioja. Le serie individuali (istogrammi grigi) aventi un correlazione più bassa di 0,4 (linea punteggiata) rispetto alla crescita media sono stati esclusi (campioni 7, 11 e 13). I dati dopo l'esclusione di questi campioni (istogrammi neri) hanno fornito una cronologia media con un migliore segnale comune di crescita, indicato da una più alta correlazione tra le rimanenti serie individuali e la cronologia media (correlazione rispettivamente pari a 0,445 e 0,526, prima e dopo l'esclusione dei campioni).
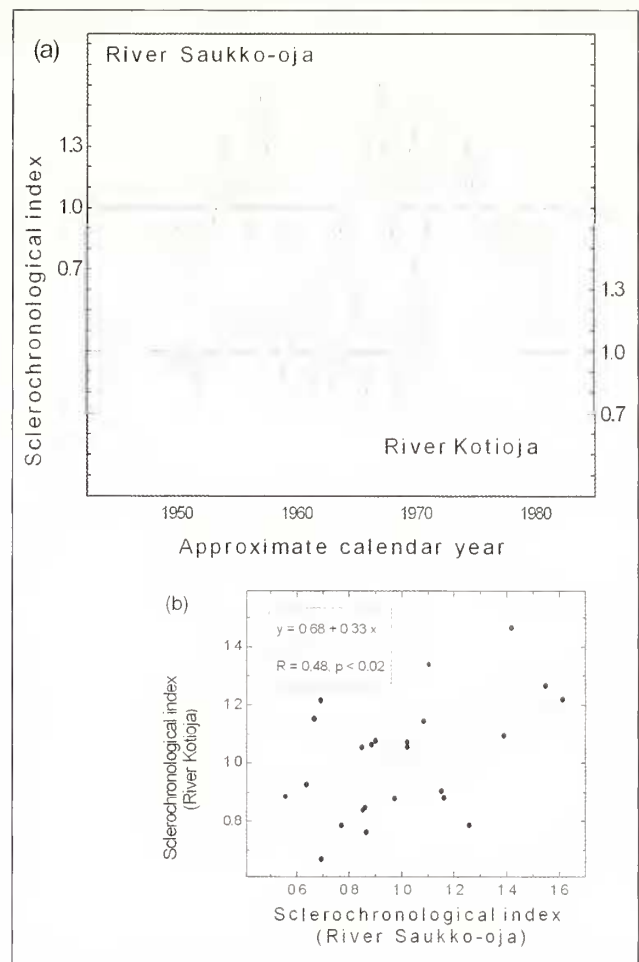
present results will be related to previous studies on the conchology of M. margaritifera.

Taphonomical interpretation implied that the shells had likely been exposed to post-mortem conditions very recently and that the valves were originally opened violently. Sclerochronological analyses showed that the mussels had died during the same calendar year. Moreover, the widths of the last increments in the studied shells were exceptionally narrow without indication of winter-line thus indicating incomplete growth during the last year of their life. These two lines of evidence, taphonomical and sclerochronological, complemented each other in pointing to a simultaneous and an abrupt death of the mussels, likely in connexion to illegal pearl hunting.

Growth variability and temporal synchrony

Prior to the temporal comparison of growth variability, the growth trends were modelled. The life-long trends, the growth trends, of the mussels show growth declines from the juvenile life-stage onwards, in some of the cases the linear regression was actually better fitted to the series than the modified negative exponential curve of Fritts et al. (1969) (**Fig. 2**). These results are consistent with the results from the internal shell growth increments of Swedish M. margaritifera (Dunca 1999) and the increments of Spanish M. margaritifera observed from the shell surface (San Miguel et al., 2004).

Comparison between the indexed shell increment series revealed considerable growth synchrony within the studied populations (**Figs. 5** and **6**). Observed synchrony implied that the growth of the mussels was influenced by same climatic and hydrological variables and that the individuals responded to external variability similarly. The shell increment growth of M. margaritifera and its correlation to climate has previously been studied among the Swedish populations (Mutvei et al., 1994; Dunca, 1999). According to Dunca et al. (2005), the annual shell growth was positively correlated with summer temperatures in Swedish rivers with decreasing climatic correlation in polluted rivers. More precisely, the highest correlation (0.59) between annual M. margaritifera shell growth increments of the compound dataset from northern to southern Sweden was found for June through August temperatures (Schöne et al. 2004) and the daily shell growth was found to co-vary with the growth season temperatures from April to October (Dunca et al. 2005). Moreover, the spatial variation in the number of sub-annual shell growth increments of M. margaritifera corresponded to number of days with water temperatures higher than + 5 °C from north to south Sweden (Dunca & Mutvei, 2001). It could be thus hypothesized that the observed shell growth variability (**Figs. 5** and **7**) is positively controlled by summer temperatures also in the present study region. Other controlling factors include food availability and the pH (Mutvei et al., 1996; Mutvei & Westermark, 2001). For example Mutvei et al. (1996) found that the annual shell

Fig. 7. Sclerochronologies for River Saukko-oja and River Kotioja; thin black line represents the total growth variability, thick grey line emphasizes the variability at decadal timescales (a). Chronologies correlated significantly when there was a 10-year lag between the last identified growth years in River Saukko-oja and River Kotioja populations (b).

Fig. 7. Sclerocronologie per il Fiume Saukko-oja ed il Fiume Kotioja. La linea sottile nera rappresenta la variabilità di crescita totale; la linea grigia spessa mette in evidenza la variabilità a scala decennale (a). Le cronologie si correlano in maniera significativa quando c'è un ritardo di 10 anni per l'ultimo incremento di crescita annuale identificato tra le popolazioni del Fiume Saukko-oja e quella del Fiume Kotioja (b).

growth increments of M. margaritifera were significantly increased after an anthropogenic widened of pH and food supply in Swedish rivers. Since the exact calendar years of the increments were unknown, the climate-growth and environment-growth correlations could not be drawn in the present study. Even without rigorous climate-growth-correlations at local-scale, the observed growth synchrony alone bears implications for the chronology construction and inter-population comparisons. The results from these approaches are discussed below.

Crossdating the increments

The presence of the growth synchrony is prerequisite for any sclerochronological study in which the principle of crossdating is applied as a tool for data quality control and relative dating of each shell growth increment (Marchitto et al., 2000; Helama et al., 2006a). In the present study, the process of crossdating was demonstrated and validated visually as well as statistically. To

quantify the agreement between the series, we used Pearson correlation coefficient as measure of common growth signal. This statistic may not be the only viable statistic to provide similar estimates but its usage has been verified previously (Marchitto et al., 2000; Helama et al., 2006a). Moreover, the common signal, as quantified by correlations between the sample series and master chronology (**Figs. 5** and **6**), was at a level comparable with those described for trees in the dendrochronological literature (see Cook & Briffa 1990). However, as already emphasized by early developers of approach, the correlations or an equivalent statistic may serve as a crossdating guide, but careful visual inspection of the samples should guide the final decision as to whether a sample is correctly crossdated (Douglass, 1941). Given that the environmental signal will likely be stronger for some sites than for others, there is no ground for setting a statistical limit for what constitutes correct cross-dating (Wigley et al. 1987).

Biochronological correlation

Subsequent to crossdating, the mean growth chronologies, sclerochronologies, were produced and compared. Comparison was carried out by the statistics of mean sensitivity, standard deviation and the first order autocorrelation. Previously in Finland, the variability (quantified both by mean sensitivity and standard deviation) of tree-ring chronologies has been shown to increase from south towards north, that is, towards harsher and more extreme growing conditions (Hustich, 1948; Mikola, 1950; Lindholm et al., 2000; Helama et al., 2005).

The two sites (River Kotioja and River Saukko-oja) are located at similar altitudes and latitudes. The similarity of the sclerochronological statistics could therefore be somewhat expected. Hastie et al. (2000) found dependence on growth characteristics of Margaritifera margaritifera and river length in Scotland. Descriptive growth parameter used by Hastie et al. (2000) ("length-at-age") was a measure of absolute growth whereas our statistics (**Tab. 1**) measured temporal growth variability. Accepting the perception of Hastie et al. (2000), that the growth characteristics of Margaritifera margaritifera could be used as river descriptives, it could be concluded that the two populations from northern Finland were growing in similar habitats, when compared by the magnitudes of environmental variability. If one aims to compare the absolute growth rates and to assess their biological relationships to the environment, more detailed information for example about the morphometrical variables would be needed. Ideally, integration of morphometrical and sclerochronological information would be preferred. This remains as target for an improved model.

Moreover, a reasonable biochronological correlation was observed for the populations that implied that the population in River Kotioja would have died ten years before the population in River Saukko-oja. Similar inter-population correlations have previously been presented for M. margaritifera in Swedish rivers, implying that the different populations exhibit parallel growth responses to regional climate (e.g. Mutvei et al., 1994; Helama et al., 2006).

If one aims to assign absolute calendar years to growth increments, it could be suggested that the presented sclerochronologies could be crossdated with live collected mussel shell growth increments or tree-rings. Whereas the collection of live specimens could be harmful for surviving populations, local tree-ring chronologies could actually provide suitable biochronological counterpart for shell growth increments. In this context, Helama et al. (2006b) were able to correlate annual shell growth increments of ocean quahog (Arctica islandica L.) and the tree-rings of Scots pine (Pinus sylvestris L.) along the coastal zone of northern Norway. This suggests that the correlation between tree-rings and freshwater shell growth increments may also be possible. Such inter-species comparison remains as future target for sclerochronologies with improved sample size and temporal length.

From conchology to conservation palaeobiology

The study of internal growth increments is a prerequisite for building reliable sclerochronologies whereas the process itself is a destructive for shells. That is why the use of museum or subfossil/fossil shells is particularly important in the case of endangered species, such as Margaritifera margaritifera. Collection of specimens alive could be especially harmful for populations that are no longer breeding. Conchological studies may help to identify the death conditions of the bivalves: fractures cutting the dorso-posterior margin may indicate violent opening of the shells by knife and the last growth increment may bear evidence about the seasonal timing of death. Variability and growth synchrony depicted by the sclerochronologies can be used as guidelines about the ecology of the species. This is to apply the principle of conservation palaeobiology (Flessa, 2002) to study the endangered species without reducing the number of individuals in the existing populations.

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References

ARAUJO R. & RAMOS A., 2001. *Action plans for* Margaritifera auricularia *and* Margaritifera margaritifera *in Europe*. Nature and Environment 117, Council of Europe, 65 pp.

BAUER G., 1986. The status of the freshwater pearl mussel *Margaritifera margaritifera* L. in the south of its European range. *Biological Conservation,* 38: 1-9.

BAUER G., 1988. Threats to the freshwater pearl mussel in Central Europe. *Biological Conservation,* 45: 239-253.

BAUER G., 1991. Plasticity in life history traits of the freshwater pearl mussel - consequences for the danger of extinction and for conservation measures. In SEITZ A. & LOESCHCKE V. (Eds.), *Species Conservation: A Population Biological Approach.* Basel, Birkhäuser Verlag, 103-120.

BAUER G., 1992. Variation in the life span and size of the freshwater pearl mussel. *Journal of Animal Ecology,* 61: 425-436.

CARROLL M., KOWALEWSKI M., SIMÕES M.G. & GOODFRIEND, G.A. 2003. Quantitative estimates of time-averaging in terebratulid brachiopod shell accumulations from a modern tropical shelf. *Paleobiology,* 29: 381-402.

COOK E.R. & BRIFFA K.R., 1990. A comparison of some treering standardization methods. In COOK E. & KAIRIUKSTIS L.A. (Eds), *Methods of Dendrochronology: Applications in the Environmental Sciences.* Dordrecht, Kluwer Academic Publishers, 153–162.

COOK E.R., BRIFFA K.R., MEKO D.M., GRAYBILL D.A. & FUNKHOUSER G., 1995. The 'segment lenght curse' in long treering chronology development for palaeoclimatic studies. *The Holocene,* 5: 229-237.

DOUGLASS A.E., 1941. Crossdating in dendrochronology. *Journal of Forestry,* 39: 825-831.

DUNCA E., 1999. Bivalve shells as archives for changes in water environment. *Vatten,* 55: 279-290.

DUNCA E. & MUTVEI H., 2001. Comparison of microgrowth pattern in *Margaritifera margaritifera* shells from south and north Sweden. *American Malacological Bulletin,* 16: 239-250.

DUNCA E., MUTVEI H. & SCHÖNE B.R., 2005. Freshwater bivalves tell of past climates: But how clearly do shells from polluted rivers speak? *Palaeogeography, Palaeoclimatology, Palaeoecology,* 228: 43-57.

FLESSA K.W., 2002. Conservation paleobiology. *American Paleontologist,* 10: 2-5.

FRITTS H.C., 1976. *Tree Rings and Climate.* Academic Press, London, 567 pp.

FRITTS H.C., SMITH D.G., CARDIS J.W. & BUDELSKY C.A., 1965. Tree-ring characteristics along a vegetational gradient in Northern Arizona. *Ecology,* 46: 393-401.

FRITTS H.C., MOSIMANN J.E. & BOTTORFF C.P., 1969. A revised computer program for standardizing tree-ring series. *Tree-Ring Bulletin,* 29: 15-20.

HASTIE L.C., YOUNG M.R. & BOON P.J., 2000. Growth characteristics of freshwater pearl mussels, *Margaritifera margaritifera* (L.). *Freshwater Biology,* 43: 243-256.

HELAMA S., LINDHOLM M., TIMONEN M. & ERONEN M., 2004. Detection of climate signal in dendrochronological data analysis: a comparison of tree-ring standardization methods. *Theoretical and Applied Climatology,* 79: 239-254.

HELAMA S., LINDHOLM M., MERILÄINEN J., TIMONEN M. & ERONEN M., 2005. Multicentennial ring-width chronologies of Scots pine along north-south gradient across Finland. *Tree-Ring Research,* 61: 21-32.

HELAMA S., SCHÖNE B.R., BLACK, B.A. & DUNCA, E., 2006a. Constructing long-term proxy series for aquatic environments with absolute dating control using a sclerochronological approach: introduction and advanced applications. *Marine and Freshwater Research,* 57: 591-599.

HELAMA S., SCHÖNE B.R., KIRCHHEFER A.J., NIELSEN J.K., RODLAND D.L., JANSSEN R., 2006b. Compound response of marine and terrestrial ecosystems to varying climate: pre-anthropogenic perspective from bivalve shell growth increments and tree-rings. *Marine Environmental Research,* 63: 185-199.

HOLMES R.L., 1983. Computer-assisted quality control in tree-ring dating and measurement. *Tree-Ring Bulletin,* 43: 69-75.

HUNTINGTON E., 1914. *The Climatic Factor as Illustrated in Arid America.* Carnegie Institution Publication 192. Carnegie Institution of Washington, Washington, 341 pp.

HUSTICH I., 1948. The Scotch pine in northernmost Finland and its dependence on the climate in the last decades. *Acta Botanica Fennica,* 42: 1-75.

JONES D.S., 1981. Annual growth increments in shells of *Spisula solidissima* record marine temperature variability. *Science,* 211: 165-167.

LINDHOLM M., LEHTONEN H., KOLSTRÖM T., MERILÄINEN J., ERONEN M. & TIMONEN M., 2000. Climatic signals extracted from ring-width chronologies of Scots pine from the Northern, Middle and Southern parts of the boreal forest belt in Finland. *Silva Fennica,* 34: 317-329.

MARCHITTO T.M., JONES G.A., GOODFRIEND G.A. & WEIDMAN C.R., 2000. Precise temporal correlation of Holocene mollusk shells using sclerochronology. *Quaternary Research,* 53: 236-246.

MIKOLA P., 1950. Puiden kasvun vaihteluista ja niiden merkityksestä kasvututkimuksissa. Summary: On the varitions in tree growth and their significance to growth studies. *Communicationes Instituti Forestalis Fenniae,* 38: 1-131.

MUTVEI H. & WESTERMARK T., 2001. How environmental information can be obtained from Naiad shells. *Ecological studies,* 145: 367-379.

MUTVEI H., WESTERMARK T., DUNCA E., CARELL B., FORBERG S. & BIGNERT A., 1994. Methods for the study of environmental changes using the structural and chemical information in molluscan shells. *Bulletin de l'Institut océanographique, Monaco, n° special,* 13: 163-186.

MUTVEI H., DUNCA E., TIMM H. & SLEPUKHINA T., 1996. Structure and growth rates of bivalve shells as indicators of environmental changes and pollution. *Bulletin de l'Institut océanographique, Monaco, n° special,* 14: 65-72.

NIELSEN J.K., 2004. Taphonomy in the light of intrinsic shell properties and life habits: marine bivalves from the Eemian of northern Russia. *Paläontologische Zeitshrift,* 78: 53-72.

SAN MIGUEL E., MONSERRAT S., FERNÁNDEZ C., AMARO R., HERMIDA M., ONDINA P. & ALTABA C.R., 2004. Growth models and longevity of freshwater pearl mussels (*Margaritifera margaritifera*) in Spain. *Canadian Journal of Zoology,* 82: 1370-1379.

SCHÖNE B.R., DUNCA E., MUTVEI H. & NORLUND U., 2004. A 217-year record of summer air temperature reconstructed from freshwater pearl mussels (*M. margaritifera,* Sweden). *Quaternary Science Review,* 23: 1803-1816.

SCHÖNE B.R., DUNCA E., FIEBIG J. & PFEIFFER M., 2005. Mutvei's solution: an ideal agent for resolving microgrowth structures of biogenic carbonates. *Palaeogeography, Palaeoclimatology, Palaeoecology,* 228: 149-166.

SEIRE A., MUTVEI H. & DUNCA E., 1993. Growth rates and structure of *Macoma balthica* shells, collected on the north coast of Estonia, as environmental indicators. Institute of Ecology, Estonian Academy of Sciences, Publication 1/1993, pp. 169-195.

SKINNER A., YOUNG M. & HASTIE L., 2003. *Ecology of the Freshwater Pearl Mussel.* Conserving Natura 2000 Rivers Ecology Series No. 2 English Nature, Peterborough, 17 pp.

TANABE K., 1988. Age and growth rate determinations of an

intertidal bivalve, *Phacosoma japonicum*, using internal shell increments. *Lethaia*, **21**: 231-241.

VALOVIRTA I., TUULENVIRE P. & ENGLUND V., 2003. *Jokihelmi-simpukan ja sen elinympäristön suojelun taso LIFE-Luonto -projektissa*. Luonnontieteellinen keskusmuseo, Finnish Museum of Natural History, Helsinki, 56 pp. [In Finnish]

WIGLEY T.M.L., JONES P.D. & BRIFFA K.R., 1987. Cross-dating methods in dendrochronology. *Journal of Archaeological Science*, **14**: 51-64.

YOUNG M.R., 1991. Conserving the freshwater pearl mussel *Margaritifera margaritifera* L. in the British Isles and continental Europe. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **1**: 73-77.

ZIUGANOV V., SAN MIGUEL E., NEVES R.J., LONGA A., FERNÁNDEZ C., AMARO R., BELETSKY V., POPKOVITCH E., KALIUZHIN S. & JOHNSON T., 2000. Life Span Variation of the Freshwater Pearl Shell: A Model Species for Testing Longevity Mechanisms in Animals. *Ambio*, **29**: 102-105.